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Acute Toxicity of Selected Substituted Phenols, Benzenes and Benzoic Acid Esters to Fathead Minnows 'Pimephales promelas'

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The Acute Toxicity of Selected Substituted Phenols, Benzenes and Benzoic Acid Esters to Fathead Minnows *Pimephales promelas*

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ABSTRACT

Flow-through acute toxicity tests were conducted with 24 organic compounds using fathead minnows Pimephales promelas *as test organisms. The tested toxicants consisted of 11 substituted phenols, four substituted benzenes and nine esters. The 96-h* LC_{50} *values determined for these compounds when tested against fathead minnows ranged from 0·135 mg litre*$^{-1}$ *for nonylphenol to 117 mg litre*$^{-1}$ *for nitrobenzene. Adverse sublethal effects were observed in fathead minnows at exposure concentrations below the 96-h* LC_{50} *value with 14 of the 24 compounds tested. Acute toxicity tests were also conducted with 2,3,4,5-tetrachlorophenol using rainbow trout* Salmo gairdneri*; with 1,4-dinitrobenzene, 2-ethoxyethylacetate, ethyl salicylate and phenyl salicylate using both channel catfish* Ictalurus punctatus *and snails* Aplexa hypnorum*; and with 4-nitrophenol using channel catfish. The 96-h* LC_{50} *for rainbow trout when tested against 2,3,4,5-tetrachlorophenol was 0·205 mg litre*$^{-1}$*, while 96-h* LC_{50} *values for channel catfish ranged from 0·673 to 44·8 mg litre*$^{-1}$ *with 1,4-dinitrobenzene and 2-ethoxyethylacetate, respectively. Snail 96-h* LC_{50} *values ranged from 4·24 mg litre*$^{-1}$ *for 1,4-dinitrobenzene to 65·2 mg litre*$^{-1}$ *for 2-ethoxyethylacetate.*

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INTRODUCTION

A series of tests was conducted to determine the acute toxicological effects produced in fathead minnows exposed to several substituted phenols, substituted benzenes and esters. The substituted phenols tested were 2-allylphenol, 2-phenylphenol, 1-naphthol, 4-tert-butylphenol, 4-tert-pentylphenol, 4-phenylazophenol, 4-amino-2-nitrophenol, 4-chloro-3-methylphenol, 2,3,4,5-tetrachlorophenol, 4-nitrophenol, and nonylphenol. The four substituted benzenes tested were naphthalene, 1-chloro-3-nitrobenzene, nitrobenzene and 1,4-dinitrobenzene. The nine esters were 2-ethoxyethylacetate, ethyl-4-aminobenzoate, methyl-2,4-dihydroxybenzoate, methyl-4-nitrobenzoate, methyl-4-chlorobenzoate, methyl-4-chloro-2-nitrobenzoate, methyl-2,5-dichlorobenzoate, ethyl salicylate and phenyl salicylate. In addition to the fathead minnow tests, acute tests were also conducted with 2,3,4,5-tetrachlorophenol using rainbow trout *Salmo gairdneri*; with 1,4-dinitrobenzene, 2-ethoxyethylacetate, ethyl salicylate and phenyl salicylate using both channel catfish *Ictalurus punctatus* and snails *Aplexa hypnorum*; and with 4-nitrophenol using channel catfish. These tests were conducted to compare sensitivity differences between fathead minnows and other freshwater species.

Many of the organic compounds tested are widely used in industry and agriculture. Knowledge of the acute effects of these compounds on freshwater organisms is important since these compounds may ultimately enter the aquatic environment where they may adversely impact the endemic organisms. The reason for using these particular compounds in this study was to provide acute toxicity data for use in an ongoing quantitative structure–activity research (QSAR) programme at the Environmental Research Laboratory-Duluth (ERL-D). With the exception of 2-ethoxyethylacetate, the chemicals tested are all benzene derivatives. Studies relating the structure to toxicity are underway as part of the ERL–D structure activity programme. The ultimate goal of the programme is to develop a model that will use certain physical properties and structural characteristics to predict the acute toxicity of untested chemicals by using the data base derived for structurally similar compounds.

The US Environmental Protection Agency neither recommends nor endorses any commercial product mentioned in this paper; trade names are used only for identification.

MATERIALS AND METHODS

Exposure system

All tests were conducted using modified proportional diluters (Mount & Brungs, 1967) with dilution factors of 0·6. Each diluter cycle delivered 2 litres of water or water plus toxicant to flow-splitting chambers (Benoit & Puglisi, 1973) which delivered 1 litre of water to randomised duplicates of five exposure concentrations plus a control. The flow-splitting chambers were modified to deliver 500 ml of water to quadruplicate tanks during all tests where both fathead minnows and channel catfish were exposed. The glass exposure tanks measured 50·0 × 14·5 × 15·0 cm deep with a 10·0 cm standpipe, which yielded a tank volume of 7·3 litres. Diluter flow rates (Table 1) were varied for the individual tests depending on the solubility rate in the saturator, and/or the stability of the compound in the test water. A constant 16-h photoperiod was used. Fluorescent lamps provided illumination, and light intensity measured 28 lm at the water surface of the exposure tanks.

Water characteristics

All tests were conducted at ERL-D. Water for all tests was obtained from Lake Superior and heated to a mean test temperature of 24·6 ± 1·4°C. Dissolved oxygen was measured once in one complete set of duplicate tanks during each test. A dissolved oxygen meter (calibrated using the azide modification of the Winkler method) was used for these analyses. The mean dissolved oxygen was 7·4 mg litre^{-1} (range 4·6–8·8 mg litre^{-1}). All other water characteristics were measured using methods described by the American Public Health Association *et al.* (1975). During each test, water hardness, alkalinity and pH determinations were made on one control or low, one intermediate, and one high concentration. Means and ranges for hardness and alkalinity were 44·9 (42·4–46·6) and 42·9 (39·6–61·4) mg litre^{-1} as $CaCO_3$, respectively, for all tests. The pH values for all tests ranged from 6·9 to 7·7.

Toxicant solutions

Nonylphenol (actual composition: 91% 4-nonylphenol, 4% 2-nonylphenol, and 5% dinonylphenol) and 4-tert-pentylphenol were obtained

TABLE 1

Purity, Diluter Flow Rates, Measured Water Concentrations (mg litre^{-1}), and Percentage Spike Recoveries for the Various Compounds Tested

Compound	*Percent purity*	*Diluter flow rates (ml min^{-1})*	*Tank No. 1 (control)*	*Tank No. 2*	*Tank No. 3*	*Tank No. 4*	*Tank No. 5*	*Tank No. 6*	*Percentage spike recoveries*
2-Allylphenol	98	100	—[a]	4·95 ± 1·54[b]	8·04 ± 2·92	13·7 ± 6·2	25·0 ± 9·3	41·2 ± 12·6	106·3 ± 4·9
2-Phenylphenol	99+	83	—[a]	1·07 ± 0·16	1·75 ± 0·24	3·01 ± 0·47	4·94 ± 0·59	8·38 ± 0·82	97·3 ± 6·7
4-Tert-butylphenol	99+	83	—[a]	1·16 ± 0·09	1·87 ± 0·15	3·10 ± 0·24	5·44 ± 0·30	9·47 ± 0·97	99·8 ± 0·5
4-Tert-pentylphenol	98+	83	—[a]	0·660 ± 0·080	1·07 ± 0·14	1·87 ± 0·25	3·34 ± 0·47	5·51 ± 0·95	96·5 ± 1·0
4-Phenylazophenol	90+	55	—[a]	0·260 ± 0·020	0·400 ± 0·040	0·630 ± 0·050	0·930 ± 0·080	1·38 ± 0·12	93·0 ± 7·2
4-Amino-2-nitrophenol	97+	55	—[a]	4·53 ± 0·43	7·15 ± 0·44	12·4 ± 0·9	21·0 ± 1·2	35·8 ± 2·3	94·8 ± 3·3
4-Chloro-3-methylphenol	99	55	—[a]	2·70 ± 0·15	4·52 ± 0·34	7·67 ± 0·45	12·9 ± 0·7	22·1 ± 1·0	102·3 ± 6·6
2,3,4,5-Tetrachlorophenol	98	55	—[a]	0·140 ± 0·013	0·277 ± 0·017	0·446 ± 0·024	0·752 ± 0·026	1·23 ± 0·06	106·3 ± 7·7
4-Nitrophenol	98	33	—[a]	9·63 ± 1·00	18·7 ± 1·3	29·9 ± 1·7	50·8 ± 2·0	89·1 ± 4·9	100·0 ± 3·2
Nonylphenol	—[c]	67	—[a]	0·023 ± 0·002	0·038 ± 0·007	0·060 ± 0·010	0·098 ± 0·013	0·187 ± 0·034	—[d]
1-Naphthol	99+	83	—[a]	1·18 ± 0·16	1·91 ± 0·25	3·29 ± 0·52	5·71 ± 1·13	9·36 ± 1·42	91·5 ± 4·1
Nitrobenzene	99	33	<2·5	31·7 ± 3·1	52·6 ± 5·2	75·4 ± 9·8	139 ± 15	255 ± 24	97·7 ± 2·5
1,4-Dinitrobenzene	98	17	—[a]	0·410 ± 0·050	0·820 ± 0·050	1·11 ± 0·19	2·73 ± 0·31	5·29 ± 0·91	84·9 ± 5·5
1-Chloro-3-nitrobenzene	98	67	—[a]	2·98 ± 0·25	4·80 ± 0·43	6·90 ± 0·80	13·7 ± 1·1	23·2 ± 1·7	95·6 ± 1·9
Naphthalene	98	67	—[a]	—[e]	—[e]	4·42 ± 0·56	5·98 ± 0·80	10·2 ± 1·0	98·9 ± 5·7
2-Ethyoxyethylacetate	99	100	—[a]	13·5 ± 0·6	21·6 ± 1·0	31·4 ± 1·1	60·3 ± 4·3	107 ± 3	100·2 ± 1·8
Ethyl-4-aminobenzoate	98	40	—[a]	5·99 ± 0·22	10·3 ± 0·1	16·1 ± 0·5	27·4 ± 0·8	46·6 ± 0·9	100·1 ± 2·1
Methyl-2,4-dihydroxybenzoate	97	40	—[a]	6·64 ± 0·15	10·3 ± 0·3	14·8 ± 0·8	27·8 ± 1·4	49·4 ± 1·0	86·2 ± 5·6
Methyl-4-nitrobenzoate	99+	7	—[a]	7·08 ± 0·82	11·5 ± 0·7	17·6 ± 1·4	30·5 ± 1·4	53·6 ± 4·6	98·9 ± 2·4
Methyl-4-chlorobenzoate	99	22	—[a]	2·69 ± 0·15	4·45 ± 0·22	6·54 ± 0·26	12·5 ± 0·2	21·8 ± 0·4	97·6 ± 3·6
Methyl-4-chloro-2-nitrobenzoate	99	100	—[a]	—[e]	—[e]	11·5 ± 2·5	19·1 ± 4·3	33·7 ± 8·0	98·3 ± 2·5
Methyl-2,5-dichlorobenzoate	99	100	<1	—[e]	—[e]	6·45 ± 1·74	11·0 ± 2·8	19·6 ± 5·1	98·7 ± 1·5
Ethyl salicylate	97	33	—[a]	2·70 ± 0·29	4·77 ± 0·46	7·61 ± 0·13	14·8 ± 1·1	25·9 ± 2·1	98·9 ± 1·2
Phenyl salicylate	96	33	—[a]	0·250 ± 0·050	0·470 ± 0·040	0·810 ± 0·020	1·94 ± 0·11	3·44 ± 0·14	92·5 ± 6·2

[a] Concentrations are not detectable.

[b] Mean and standard deviation.

[c] The actual composition of nonylphenol was 91% 4-nonylphenol, 4% 2-nonylphenol and 5% 2,4-dinonylphenol.

[d] For this chemical a method of standard additions was used and therefore no recoveries are available.

[e] These concentrations were deleted so the saturator could maintain the remaining concentrations with more uniformity.

from the Pfalz and Bauer Chemical Company, Stamford, CT, 4-nitrophenol was obtained from the Eastman Chemical Company, Rochester, NY, and all other tested chemicals were obtained from the Aldrich Chemical Company, Milwaukee, WI. The percent purity and measured water concentrations in the tanks for all chemicals tested are given in Table 1. All the tested compounds, with the exception of 4-nitrophenol and 2-ethoxyethylacetate, have low solubility in water. For this reason, a saturator system similar to the one described by Phipps *et al.* (1982) was used to generate saturated solutions of all low-solubility compounds. The saturator consisted of two to six 19-litre stainless steel soda carbonation vessels that were connected in series. With 11 of the 22 compounds tested the carbonation vessels were also heated in a water bath to increase the solubility of the compound in the saturator. The contents of all cans were also stirred using a magnetic stir bar. Depending on the solubility and toxicity of each compound, a predetermined amount of dissolved compound in water was pumped to the diluter during each cycle using a metering pump controlled by an automatic timer. The 4-nitrophenol and 2-ethoxyethylacetate were dissolved in individual soda carbonation vessels, and precalculated amounts of these solutions were pumped into the mix cell of the diluter during each cycle.

Toxicant concentrations were analysed four times during all the 96-h exposures. Daily water samples for chemical analysis were taken at mid-depth of each tank from one set of duplicates (six tanks).

Chemical methods

The following compounds were analysed by the automated 3-methyl-2-benzothiazolone hydrazone (MBTH) method (Friestad *et al.*, 1969) as modified by Gales (1975): 2-allylphenol, 2-phenylphenol, 1-naphthol, 4-tert-butylphenol, 4-tert-pentylphenol, 4-phenylazophenol, 4-amino-2-nitrophenol, 4-chloro-3-methylphenol and 4-nitrophenol. The distillation step was found to be unnecessary and was therefore eliminated.

Nonylphenol and naphthalene were analysed using a Baird Atomic Ratio Recording Spectrofluorimeter (Model SFR100). For nonylphenol the wavelength settings were 284 nm for excitation and 307 nm for emission; the slit widths used were 20 nm for both excitation and emission; samples were extracted (1:1) with hexane and analysed using standards prepared in hexane. For naphthalene the emission and excitation wavelengths were 265 and 334 nm, respectively; emission and

excitation slit widths were 10 and 20 nm, respectively; samples and standards were analysed directly in Lake Superior water after adjusting to contain 25% 2-propanol.

The following compounds were analysed by ultraviolet spectroscopy using a Beckman Model DB-G spectrophotometer: ethyl salicylate, phenyl salicylate, ethyl-4-aminobenzoate, methyl-2,4-dihydroxybenzoate, methyl-4-nitrobenzoate, methyl-4-chlorobenzoate, methyl-4-chloro-2-nitrobenzoate and methyl-2-5-dichlorobenzoate. A wavelength scan of each compound was done to determine the optimal wavelength for analysis. The spectrophotometer was zeroed on distilled water and samples were corrected for the absorbance of the exposure water blank.

The 2,3,4,5-tetrachlorophenol was analysed by gas chromatography using a Hewlett–Packard 5730A automatic gas chromatograph equipped with a Model 3552A data system and a ^{63}Ni electron capture detector. The column was packed with 100/120 mesh Supelcoport coated with 1·5% SP2250/1·95% SP-2401. The carrier gas was 5% methane in argon and the column temperature was 160 °C. Retention time was about 4·0 min. Water samples were added directly to 100-ml volumetric flasks to which 50 ml of hexane and 0·1 ml of concentrated H_2SO_4 had already been added. The samples were then stirred vigorously on magnetic stirring devices for 1·5 h and allowed to separate for 1·0 h. Hexane extract samples (1·0 ml) were then added to GC vials followed by 0·1 ml of diazoethane derivatising agent.

Compounds 1-chloro-3-nitrobenzene and nitrobenzene were analysed using a Hewlett–Packard model 5730A gas chromatograph equipped with a flame ionisation detector. The glass column was 1·8 m × 2 mm internal diameter packed with 10% carbowax 20M on 80/100 mesh Gas Chrom Q. Nitrogen was the carrier gas at a flow rate of 30 ml min^{-1}. Hydrogen and air flow rates were 25 and 240 ml min^{-1}, column temperatures were 190 and 165 °C and retention times were 3·94 and 4·68 min for 1-chloro-3-nitrobenzene and nitrobenzene, respectively. 1-Chloro-3-nitrobenzene was extracted with ether:hexane (25:75) and nitrobenzene was extracted with hexane in a volumetric flask by stirring vigorously on a magnetic stirrer.

The 1,4-dinitrobenzene was extracted as described for 1-chloro-3-nitrobenzene. Analysis was performed on a Tracor Model 550 gas chromatograph equipped with a ^{63}Ni electron capture detector. The column was 1·8 m × 2 mm internal diameter and packed with 1·5% OV-17/1·95% QF1 on 80/100 mesh Gas Chrom Q. The carrier gas was

argon:methane (95:5) with a flow rate of 50 ml min^{-1}. The column was operated at 180 °C and 1,4-dinitrobenzene had a retention time of 2·21 min.

Analysis of 2-ethoxyethylacetate was by gas chromatography using a Hewlett–Packard Model 5730A gas chromatograph equipped with a flame ionisation detector. Aqueous samples were injected without treatment. The column was 0·9 × 2 mm internal diameter packed with 60/80 mesh Tenax GC. Nitrogen was the carrier gas at a flow rate of 25 ml min^{-1}. Hydrogen and air flow rates were 25 and 240 ml min^{-1}, respectively. The column was operated isothermally at 150 °C. The retention time of 2-ethoxyethylacetate was 4·0 min.

All sample concentrations were calculated by linear regression analysis using standard curves of at least three points.

Biological procedures

Fathead minnow tests were conducted using 31- through 35-day-old fish which were obtained as newly hatched larvae from the stock culture unit of ERL-D. Larvae were kept in a rearing system at 25 °C and were fed live brine shrimp (Bio-Marine Research Inc., Hawthorne, CA) until used for testing. Adult snails were obtained from a culture maintained in the rearing system used for fathead minnows. Juvenile rainbow trout (Fattig Hatcheries, Brady, NE) and channel catfish (Senecaville National Fish Hatchery, Senecaville, OH) were held in 530-litre fibreglass holding tanks at 14 and 23 °C, respectively, and fed a commercial formulation of dry fish food until used for testing.

Fifty fathead minnows (25 per duplicate tank) were exposed in each concentration and in controls for all tests except with nonylphenol. Due to an insufficient supply of test fish only 40 fatheads (20 per duplicate tank) were used in the nonylphenol acute. Average weights of exposed fish were obtained by weighing subsamples of 25 fathead minnows (Table 2), and 20 rainbow trout and channel catfish (Table 3). The numbers of rainbow trout, channel catfish and snails exposed per concentration are given in Table 3. Snails were counted into stainless steel screen cylinders 10 × 3 cm wide which were closed on one end by a stainless steel screen and on the other end by a No. 7 neoprene stopper. The cylinders were then randomly distributed to the exposure tanks which also contained fathead minnows. Groups of fish were counted into plastic freezer containers and were randomly assigned to the exposure tanks. Fish used

for testing were not fed 24 h before or during a test. Death was the primary endpoint, although effects on fish equilibrium, behaviour, coloration, respiration, and deformities were also noted. Dead fish were counted and removed three times on the first day and twice daily thereafter. Snail mortalities were counted at the end of the 96-h tests.

Determinations of LC_{50} values were made using a computerised Trimmed Spearman–Karber method (Hamilton *et al.*, 1977). Mortality data from the duplicate exposure tanks were combined before LC_{50} determinations were conducted. In cases where the Trimmed Spearman–Karber method could not give 95% confidence limits for the LC_{50} values, confidence limits were calculated using the binomial test as described by Stephan (1977).

Species sensitivity factors were derived by dividing the 96-h LC_{50} value of the least sensitive species by the 96-h LC_{50} for the most sensitive species. Other acute procedures or methods not specified followed those described by the US Environmental Protection Agency (1975).

RESULTS

The LC_{50} values for fathead minnows are shown in Table 2 and for trout, channel catfish and snails in Table 3. Ethyl salicylate and phenyl salicylate concentrations of 25·9 and 3·44 mg litre^{-1}, respectively, killed less than 50% of the exposed snails, and the LC_{50} values for these compounds are reported as greater than the highest exposure concentrations.

Fish sensitivity differences were quite small (range of 1·1–2·7 times) for the two substituted phenols, one substituted benzene and the three esters tested with more than one species. Rainbow trout were slightly more than twice as sensitive to 2,3,4,5-tetrachlorophenol than fathead minnows. Channel catfish were approximately 1·1 times less sensitive than fathead minnows to 2-ethoxyethylacetate, phenyl salicylate and 1,4-dinitrobenzene. However, catfish were approximately 1·3 and 2·7 times more sensitive than fathead minnows to ethyl salicylate and 4-nitrophenol, respectively. In all snail tests, snails were less sensitive than either fathead minnows or channel catfish. At 96 h, snails were 1·5 and 7·0 times less sensitive than fathead minnows to 2-ethoxyethylacetate and 1,4-dinitrobenzene, respectively.

The behavioural and physiological changes observed in fathead

TABLE 2

Size of Exposed Fathead Minnows and the 24-, 48-, 72- and 96-h LC_{50} Values (mg litre^{-1}) for the Various Compounds Tested

Toxicant	*Mean weight of exposed fatheads (mg)*	*24-h LC_{50}*	*48-h LC_{50}*	*72-h LC_{50}*	*96-h LC_{50}*
2-Allylphenol	86	34·1 (30·4–38·1)[a]	17·8 (16·2–19·6)	14·3 (13·2–15·5)	13·2 (12·1–14·4)
2-Phenylphenol	110	6·24 (6·03–6·46)	6·11 (5·85–6·38)	6·11 (5·85–6·38)	5·99 (5·70–6·30)
4-Tert-butylphenol	97	6·21 (5·78–6·68)	5·69 (5·23–6·18)	5·26 (4·81–5·75)	5·14 (4·71–5·62)
4-Tert-pentylphenol	102	3·17 (2·94–3·42)	2·58 (2·49–2·68)	2·50 (1·87–3·34)[b]	2·50 (1·87–3·34)[b]
4-Phenylazophenol	100	—[c]	—[c]	1·10 (1·07–1·13)	1·09 (1·05–1·13)
4-Amino-2-nitrophenol	150	—[c]	—[c]	—[c]	34·3 (29·9–39·4)
4-Chloro-3-methylphenol	106	13·3 (12·1–14·5)	11·4 (10·3–12·8)	9·21 (7·88–10·8)	7·56 (6·41–8·92)
2,3,4,5-Tetrachlorophenol	—	0·496 (0·462–0·534)	0·450 (0·418–0·485)	0·441 (0·410–0·475)	0·441 (0·410–0·475)
4-Nitrophenol	84	64·6 (58·4–71·5)	54·4 (49·7–59·5)	44·1 (40·7–47·8)	41·0 (37·7–44·6)
Nonylphenol	220	—[c]	0·164 (0·145–0·186)	0·137 (0·134–0·140)	0·135 (0·098–0·187)[b]
1-Naphthol	114	7·01 (6·74–7·30)	4·33 (3·29–5·71)[b]	4·24 (4·12–4·37)	4·24 (4·12–4·37)
Nitrobenzene	160	163 (151–175)	156 (144–170)	127 (114–140)	117 (105–130)
1,4-Dinitrobenzene	164	0·687 (0·642–0·734)	0·641 (0·605–0·678)	0·609 (0·584–0·636)	0·603 (0·581–0·627)
1-Chloro-3-nitrobenzene	148	18·0 (17·7–18·3)	18·0 (17·7–18·3)	18·0 (17·7–18·3)	18·0 (17·7–18·3)
Naphthalene	116	7·76 (7·39–8·14)	6·35 (5·95–6·77)	6·08 (5·74–6·44)	6·08 (5·74–6·44)
2-Ethoxyethylacetate	148	44·0 (43·0–45·1)	43·5 (31·4–60·3)[b]	42·6 (41·4–43·8)	42·2 (40·7–43·6)
Ethyl-4-aminobenzoate	104	35·4 (34·6–36·1)	35·4 (34·6–36·1)	35·4 (34·6–36·1)	35·4 (34·6–36·1)
Methyl-2,4-dihydroxybenzoate	104	41·4 (38·6–44·5)	38·5 (37·3–39·9)	38·5 (37·3–39·9)	38·5 (37·3–39·9)
Methyl-4-nitrobenzoate	123	23·8 (22·4–25·4)	23·8 (22·4–25·4)	23·6 (22·2–25·0)	23·6 (22·2–25·0)
Methyl-4-chlorobenzoate	109	14·6 (11·0–19·5)	12·5 (10·9–14·4)	10·9 (9·68–12·2)	10·9 (9·68–12·2)
Methyl-4-chloro-2-nitrobenzoate	280	29·4 (26·9–32·1)	28·0 (26·3–29·9)	28·0 (26·3–29·9)	27·2 (25·9–28·6)
Methyl-2,5-dichlorobenzoate	184	14·4 (13·9–14·8)	13·9 (13·3–14·7)	13·9 (13·3–14·7)	13·8 (13·1–14·7)
Ethyl salicylate	132	19·8 (19·5–20·1)	19·7 (19·5–19·9)	19·6 (14·8–25·9)[b]	19·6 (14·8–25·9)[b]
Phenyl salicylate	184	1·15 (1·06–1·25)	1·09 (0·990–1·20)	1·09 (0·990–1·20)	1·09 (0·990–1·20)

[a] LC_{50} values and their respective 95% confidence intervals.
[b] Confidence intervals (>99·9%) were calculated for these time periods using the binomial test.
[c] No LC_{50} value could be determined because of insufficient mortality in the highest exposure concentrations.

TABLE 3

The 24-, 48-, 72- and 96-h LC_{50} Values (mg litre^{-1}) for Various Species Tested in Conjunction with the Fathead Minnows

Toxicant	*Species*	*Size or life stage*	*Number of organisms exposed per concentration*	*24-h LC_{50}*	*48-h LC_{50}*	*72-h LC_{50}*	*96-h LC_{50}*
2,3,4,5-Tetrachlorophenol	Rainbow trout	10·4 gr	20	0·304 (0·277–0·334)[a]	0·284 (0·180–0·447)[b]	0·284 (0·180–0·447)[b]	0·205 (0·172–0·245)
4-Nitrophenol	Channel catfish	0·5 gr	30	15·0 (14·0–16·0)	15·0 (14·0–16·0)	15·0 (14·0–16·0)	15·0 (14·0–16·0)
1,4-Dinitrobenzene	Channel catfish	0·5 gr	20	0·899 (0·782–1·03)	0·822 (0·742–0·910)	0·725 (0·649–0·810)	0·673 (0·608–0·746)
1,4-Dinitrobenzene	Snail[c]	Adults	20	—	—	—	4·24 (3·79–4·75)
2-Ethoxyethylacetate	Channel catfish	3·4 gr	20	80·1 (60·3–107)[b]	60·9 (53·1–69·7)	44·8 (42·2–47·6)	44·8 (42·2–47·6)
2-Ethoxyethylacetate	Snail[c]	Adults	20	—	—	—	65·2 (57·0–74·5)
Ethyl salicylate	Channel catfish	4·6 gr	20	20·6 (19·3–21·9)	19·0 (17·9–20·2)	16·3 (14·4–18·5)	14·9 (13·0–17·0)
Ethyl salicylate	Snail[c]	Adults	20	—	—	—	>25·9
Phenyl salicylate	Channel catfish	4·6 gr	20	1·30 (1·21–1·39	1·25 (0·810–1·94)[b]	1·25 (0·810–1·94)[b]	1·25 (0·810–1·94)[b]
Phenyl salicylate	Snail[c]	Adults	20	—	—	—	>3·44

[a] LC_{50} values and their respective 95% confidence intervals.
[b] Confidence intervals (>99·9%) were calculated for these time periods using the binomial test.
[c] Only 96-h mortality counts were made with snails.

TABLE 4

The Lowest Concentration (mg litre^{-1}) where Behavioural and Physiological Changes were Observed in Fathead Minnows Exposed to Various Compounds during the 96-h Acute Toxicity Tests

Compound	*Observed effect*[a]								
	Narcotised—fish unreactive to outside stimuli[b]	*Lethargic—fish still reactive to outside stimuli*[b]	*Fish swimming at water surface*	*Hyperactivity—fish darting and rolling*	*Some loss of equilibrium*	*Haemorrhaged areas*	*Deformities*	*Changes in coloration*	*Bodies swollen with fluid*
2-Allylphenol	—	13·7	4·95	—	13·7	—	13·7	8·04	—
2-Phenylphenol	—	4·94	—	—	8·38	—	—	—	—
4-Tert-butylphenol	3·10	—	—	—	—	—	5·44	—	—
4-Tert-pentylphenol	1·87	—	—	—	3·34	—	3·34	—	—
4-Phenylazophenol	—	—	—	1·38	0·930	—	—	—	—
4-Amino-2-nitrophenol	—	—	21·0	21·0	35·8	—	—	—	—
4-Chloro-3-methylphenol	—	—	2·70	22·1	4·52	—	—	—	—
2,3,4,5-Tetrachlorophenol	—	—	—	—	0·752	—	—	—	—
4-Nitrophenol	50·8	—	—	—	—	—	—	—	—
Nonylphenol	—	0·187	—	—	0·098	0·187	—	—	0·187
1-Naphthol	—	—	—	9·36	5·71	—	—	—	—
Nitrobenzene	139	52·6	—	—	75·4	—	—	—	—
1,4-Dinitrobenzene	—	2·73	—	—	1·11	—	—	—	—
1-Chloro-3-nitrobenzene	13·7	6·90	—	—	13·7	—	—	—	—
Naphthalene	—	4·42	—	—	5·98	—	5·98	—	—
2-Ethoxyethylacetate	107	—	—	—	—	—	—	—	—
Ethyl-4-aminobenzoate	27·4	16·1	—	—	46·6	—	—	—	—
Methyl-2,4-dihydroxybenzoate	—	—	—	27·8	49·4	—	—	—	—
Methyl-4-nitrobenzoate	—	30·5	53·6	—	30·5	—	—	—	—
Methyl-4-chlorobenzoate	—	6·54	6·54	—	6·54	—	—	—	—
Methyl-4-chloro-2-nitrobenzoate	33·7	19·1	—	—	11·5	19·1	11·5	—	—
Methyl-2,5-dichlorobenzoate	—	6·45	—	—	11·0	—	—	—	—
Ethyl salicylate	7·61	—	14·8	—	25·9	—	—	—	—
Phenyl salicylate	—	—	1·94	1·94	1·94	—	—	—	—

[a] These observed effects were variable with time, but generally involved the majority of fish at the concentration listed; however, effects on loss of equilibrium, haemorrhaged areas and deformities were noted in cases where more than one fish was affected.

[b] Outside stimuli consisted of tapping the outside of the tank and observing the response of the fish.

minnows during exposures to the 24 organic compounds tested are given in Table 4. With the range of concentrations tested in the present study, nonylphenol and methyl-4-chloro-2-nitrobenzoate produced haemorrhaged areas in fathead minnows, and methyl-4-chloro-2-nitrobenzoate, 2-allylphenol, 4-tert-butylphenol, 4-tert-pentylphenol and naphthalene caused slight to severe cases of spinal deformities. Some loss of equilibrium was noted in fathead minnows exposed to toxicant concentrations below the 96-h LC_{50} values with 4-phenylazophenol, 4-chloro-3-methylphenol, nonylphenol, nitrobenzene, 1-chloro-3-nitrobenzene, naphthalene, methyl-4-chlorobenzoate, methyl-4-chloro-2-nitrobenzoate and methyl-2,5-dichlorobenzoate.

DISCUSSION

Sublethal effects such as lethargy, loss of equilibrium and body deformities could produce a dramatic environmental impact on a fish species due to increased susceptibility to predation and/or inability to obtain food. For these reasons, fish which have lost equilibrium would not be expected to survive such toxicant exposures outside laboratory conditions. Fathead minnows in the present tests were observed to be narcotised and lethargic, to have some loss of equilibrium and/or to have body deformities at exposure concentrations below the 96-h LC_{50} value for 14 of the 24 tested chemicals, namely: 2-phenylphenol, 4-tert-butylphenol, 4-tert-pentylphenol, 4-phenylazophenol, 4-chloro-3-methylphenol, nonylphenol, nitrobenzene, 1-chloro-3-nitrobenzene, naphthalene, ethyl-4-aminobenzoate, methyl-4-chlorobenzoate, methyl-4-chloro-2-nitrobenzoate, methyl-2,5-dichlorobenzoate and ethyl salicylate. Thus deleterious effects on fathead minnow populations can be expected at concentrations of these compounds somewhat below the LC_{50} level.

There is a general lack of acute toxicity information on the compounds reported here for freshwater fish. Only papers containing 96-h toxicity information will be used for comparisons.

The 96-h LC_{50} of 4-nitrophenol for fathead minnows was 41·0 mg litre^{-1}, which lies between the 96-h LC_{50} values of 8·3 and 60·5 mg litre^{-1} determined previously for 4-nitrophenol in bluegills *Lepomis macrochirus* (Buccafusco *et al.*, 1981) and in fathead minnows (Phipps *et al.*, 1981), respectively.

The fathead minnow 96-h LC_{50} for 4-chloro-3-methylphenol was 7·56 mg litre^{-1} in the present study. This LC_{50} value is much higher than the corresponding value of 0·03 mg litre^{-1} previously reported (US Environmental Protection Agency, 1972) for 4-chloro-3-methylphenol under static conditions. However, this latter LC_{50} was extrapolated from 80 and 100% mortality at 1 and 10·0 mg litre^{-1}, respectively—a difficult task.

Buccafusco *et al.* (1981) determined a bluegill 96-h LC_{50} of 43·0 mg litre^{-1} for nitrobenzene, which is less than half of the value calculated during the present test for fathead minnows.

Nonylphenol, the most toxic compound tested in these experiments, had a fathead minnow 96-h LC_{50} of 0·135 mg litre^{-1} which is in the range of values (0·13–0·19 mg litre^{-1}) reported for juvenile Atlantic salmon *Salmo salar* (McLeese *et al.*, 1981) but is less than the value (0·90 mg litre^{-1}) reported in earlier work (McLeese *et al.*, 1980).

The present 96-h LC_{50} of 6·08 mg litre^{-1} for naphthalene for fathead minnows lies between the 96-h LC_{50} values of 3·22 and 150 mg litre^{-1} for coho salmon fry *Oncorhynchus kisutch* (Moles, 1980) and mosquitofish *Gambusia affinis* in turbid water (Wallen *et al.*, 1957), respectively.

Present testing produced a fathead minnow 96-h LC_{50} of 18·0 mg litre^{-1} for 1-chloro-3-nitrobenzene, while Dawson *et al.* (1975/77) found a bluegill static 96-h LC_{50} of 1·2 mg litre^{-1} for this compound.

Dawson *et al.* (1975/77) also tested 2-ethoxyethylacetate with bluegills and found a 96-h LC_{50} of 45·0 mg litre^{-1} which is very close to the present fathead minnow value of 42·2 mg litre^{-1}. With the exception of 2-ethoxyethylacetate, bluegills appeared to be somewhat more sensitive than fathead minnows when exposed to compounds tested in the present study.

No 96-h toxicity information on freshwater fish was found that dealt with the remaining compounds currently tested.

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REFERENCES

American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). *Standard methods for the examination of water and wastewater*, 14th edn. New York, American Public Health Association.

Benoit, D. A. & Puglisi, F. A. (1973). A simplified flow-splitting chamber and siphon for proportional diluters. *Water Res.*, **7**, 1915–16.

Buccafusco, R. J., Ells, S. J. & LeBlanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus*). *Bull. environ. Contam. & Toxicol.*, **26**, 446–52.

Dawson, G. W., Jennings, A. L., Drozdowski, D. & Rider, E. (1975/77). The acute toxicity of 47 industrial chemicals to fresh and saltwater fishes. *J. Hazard. Mater.*, **1**, 303–18.

Friestad, H. O., Otti, E. E. & Ganther, F. A. (1969). Automated colorimetric micro determination of phenol by oxidative coupling with 3-methyl-2-benzothiazolone hydrazone. *Technicon International Congress, 4–6 June 1969, Chicago, Illinois*, 21–7.

Gales, M. E. (1975). An evaluation of the 3-methyl-2-benzothiazolone hydrazone method for the determination of phenols in water and waste waters. *Analyst, Lond.*, **100**, 841–7.

Hamilton, M. A., Russo, R. C. & Thurston, R. V. (1977). Trimmed Spearman-Karber method for estimated median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.*, **7**, 714–19. Correction, **12**, 417 (1978).

McLeese, D. W., Zitko, V., Metcalfe, C. D. & Sergeant, D. B. (1980). Lethality of aminocarb and the components of the aminocarb formulation to juvenile Atlantic salmon, marine invertebrates and a freshwater clam. *Chemosphere*, **9**, 79–82.

McLeese, D. W., Zitko, V., Sergeant, D. B., Burridge, L. & Metcalfe, C. D. (1981). Lethality and accumulation of alkylphenols in aquatic fauna. *Chemosphere*, **10**, 723–30.

Moles, A. (1980). Sensitivity of parasitized coho salmon fry to crude oil, toluene, and naphthalene. *Trans. Am. Fish. Soc.*, **109**, 293–6.

Mount, D. I. & Brungs, W. A. (1967). A simplified dosing apparatus for fish toxicology studies. *Water Res.*, **1**, 21–9.

Phipps, G. L., Holcombe, G. W. & Fiandt, J. T. (1981). Acute toxicity of phenol and substituted phenols to the fathead minnow. *Bull. environ. Contam. & Toxicol.*, **26**, 585–93.

Phipps, G. L., Holcombe, G. W. & Fiandt, J. T. (1982). Saturator system for generating toxic water solutions for aquatic bioassays. *Progrve Fish-Cult.*, **44**, 115–16.

Stephan, C. E. (1977). Methods for calculating an LC_{50}. In *Aquatic toxicology and hazard evaluation*, ed. by F. L. Mayer and L. Hamelink, 65–84. American Society for Testing and Materials, ASTM STP 634.

US Environmental Protection Agency (1972). The effect of chlorination on

selected organic chemicals. *Water Pollution Control Research Series*, **12020**, 73–81.

US Environmental Protection Agency (1975). *The committee on methods for toxicity tests with aquatic organisms. Methods for acute toxicity tests with fish, macroinvertebrates and amphibians*. EPA-660/3-75-009, Duluth, MN, US Environmental Protection Agency.

Wallen, I. E., Greer, W. C. & Lasater, R. (1957). Toxicity to *Gambusia affinis* of certain pure chemicals in turbid waters. *Sewage & Industrial Wastes*, **29**, 695–711.